

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

LISTING OF CLAIMS

1. (Currently amended) A method of detecting a target nucleotide sequence in a sample, comprising:
 - a) providing
 - i) template comprising at least one target nucleotide sequence;
 - ii) at least one probe oligonucleotide comprising a 3' portion complementary to a portion of said template comprising target nucleotide sequence and a 5' portion not complementary to said template comprising target nucleotide sequence;
 - iii) at least one upstream oligonucleotide complementary to a portion of said template comprising target nucleotide sequence wherein said portion is 5' 3' to and partially overlapping the 3' portion of said template comprising target nucleotide sequence complementary to said probe oligonucleotide; and
 - iv) a cleaving agent;
 - b) mixing, in any order, said template comprising target nucleotide sequence, said probe oligonucleotide, said upstream oligonucleotide, and said ~~cleavage means~~ cleaving agent under reaction conditions such that said 3' portion of said probe oligonucleotide is annealed to said template and said upstream oligonucleotide is annealed to said template so as to create a cleavage structure wherein said probe oligonucleotide and said upstream oligonucleotide overlap by at least one nucleotide;
 - c) cleaving said cleavage structure to release a 5' flap, comprising cleaving said probe oligonucleotide at a position one nucleotide 3' of the portion of said probe oligonucleotide that overlaps said upstream oligonucleotide, releasing said 5' flap comprising said 5' portion of said probe oligonucleotide not complementary to contiguous target nucleotide sequence and further comprising any overlapping nucleotide;

d) utilizing said 5' flap as a reagent in at least one subsequent reaction to generate at least one tagged molecule comprising an identifier tag chosen to serve as an identifier for said target nucleotide sequence;

e) contacting said at least one tagged molecule with a universal detector comprising at least one complementary detection probe coupled to a detection means, wherein said complementary detection probe comprises sequence complementary to said identifier tag; and

f) measuring hybridization of said identifier tag to said complementary detection probe, wherein said hybridization indicates the presence of the corresponding target nucleotide sequence in said sample.

2. (Original) The method of Claim 1, wherein said template is DNA or RNA.

3. (Original) The method of Claim 2, wherein said template comprises any one of genomic DNA, polymerase chain reaction (PCR) product, cDNA, rolling circle (RC) amplification product, mRNA, or viral RNA.

4. (Original) The method of Claim 1, wherein said reaction conditions comprise a reaction temperature between approximately 40 and approximately 75 degrees Centigrade.

5. (Original) The method of Claim 1, wherein multiple probe oligonucleotides are cleaved and multiple 5' flaps are released.

6. (Original) The method of Claim 1, wherein said method is used to detect variant sequences of said target nucleotide sequence.

7. (Original) The method of Claim 1, wherein said probe oligonucleotide and said upstream oligonucleotide overlap by one nucleotide.

8. (Original) The method of Claim 7, wherein said method is used to determine one or more polymorphic nucleotides in a single nucleotide polymorphism (SNP), said method comprising providing at least one probe oligonucleotide and at least one upstream oligonucleotide complementary to and overlapping at the polymorphic nucleotide of a first allele of said SNP, and further providing at least one probe oligonucleotide and at least one upstream oligonucleotide complementary to and overlapping at the polymorphic nucleotide of a second allele of said SNP, wherein said oligonucleotide probes and upstream oligonucleotides anneal to said template so as to create a distinct cleavage structure for each allele of said SNP, with the

result that an allele-specific 5' flap is released from each cleavage structure if the corresponding allele of said SNP is present in said sample and with the further result that each said allele-specific 5' flap released from said cleavage structure generates an allele-specific tagged molecule, wherein each said allele-specific tagged molecule comprises the identifier tag chosen to serve as the identifier for the corresponding allele.

9. (Original) The method of Claim 8, wherein said SNP comprises more than two alleles, further comprising providing oligonucleotide probe and upstream oligonucleotides complementary to and overlapping at each said allele of said SNP.

10. (Original) The method of Claim 1, wherein a plurality of target nucleotide sequences in a sample are detected, said method comprising:

a) providing template comprising a plurality of target nucleotide sequences, and further providing at least one probe oligonucleotide and at least one upstream oligonucleotide complementary to a portion of template comprising each target nucleotide sequence of said plurality of target nucleotide sequences;

b) mixing said oligonucleotide probes and upstream oligonucleotides with said template under reaction conditions such that each oligonucleotide probe and upstream oligonucleotide will anneal to said template to create a distinct cleavage structure for each target nucleotide sequence, with the result that at least one distinct 5' flap corresponding to each said target nucleotide sequence of said plurality of target nucleotide sequences is released from each distinct cleavage structure if the corresponding target nucleotide sequence is present in said sample;

c) utilizing each said distinct 5' flap as a reagent in at least one subsequence reaction to generate at least one distinct tagged molecule corresponding to each said target nucleotide sequence of said plurality of target nucleotide sequences, wherein each said distinct tagged molecule comprises the identifier tag chosen to serve as an identifier to each corresponding target nucleotide sequence; and

d) measuring hybridization of each said identifier tag corresponding to each said target nucleotide sequence of said plurality of target nucleotide sequences, wherein each said hybridization indicates the presence of said corresponding target nucleotide sequence in said sample.

11. (Original) The method of Claim 1, wherein said cleaving agent is a 5' endonuclease.

12. (Currently amended) A method of detecting a target nucleotide sequence in a sample, comprising:

- a) performing a first ICR invasion cleavage reaction (ICR) as in steps a) to c) of Claim 1, releasing a first 5' flap;
- b) performing a second ICR invasion cleavage reaction (ICR) comprising:
 - i) providing a second template, a second probe oligonucleotide, and said first 5' flap, wherein said second probe oligonucleotide comprises a 3' portion complementary to a portion of said second template and a 5' portion not complementary to said second template, and further wherein said first 5' flap is 5' to and partially overlapping the 3' portion of said second template comprising target nucleotide sequence complementary to said second probe oligonucleotide;
 - ii) mixing, in any order, said second template, said second probe oligonucleotide, and said first 5' flap, under reaction conditions such that said 3' portion of said second probe oligonucleotide is annealed to said second template and said first 5' flap is annealed to said second template so as to create a cleavage structure wherein said second probe oligonucleotide and said first 5' flap overlap by at least one nucleotide;
 - iii) cleaving said cleavage structure to release a second 5' flap, comprising cleaving said second probe oligonucleotide at a position one nucleotide 3' of the portion of said second probe oligonucleotide that overlaps said first 5' flap, releasing said second 5' flap comprising said 5' portion of said second probe oligonucleotide not complementary to contiguous target nucleotide sequence and further comprising any overlapping nucleotide;
- c) utilizing said second 5' flap as a reagent in at least one subsequent reaction to generate at least one tagged molecule comprising an identifier tag chosen to serve as an identifier for said target nucleotide sequence;
- d) contacting said at least one tagged molecule with a universal detector comprising at least one complementary detection probe coupled to a detection means,

wherein said complementary detection probe comprises sequence complementary to said identifier tag; and

e) measuring hybridization of said identifier tag to said complementary detection probe, wherein said hybridization indicates the presence of the corresponding target nucleotide sequence in said sample.

13. (Currently amended) The method of Claim 12, wherein no additional ~~cleavage means~~ cleaving agent is provided for said second ICR.

14. (Currently amended) The method of Claim 12, wherein an additional ~~cleavage means~~ cleaving agent is provided for said second ICR.

15. (Original) The method of Claim 12, wherein said second template and said second probe oligonucleotide are provided as a hairpin cassette.

16. (Original) The method of Claim 15, wherein said hairpin cassette is addressably labelled, such that said labelled hairpin cassette can be manipulated through a label-binding moiety.

17. (Original) The method of Claim 16, wherein said hairpin cassette is biotinylated.

18. (Original) The method of Claim 17, wherein said biotinylated hairpin cassette is contacted with streptavidin coupled to a solid support, such that said biotinylated hairpin cassette can be removed from the reaction mixture.

19. (Original) The method of Claim 12, wherein said tagged molecule comprises an identifier tag chosen to serve as an identifier for said target nucleotide sequence, and a development reagent.

20. (Original) The method of Claim 19, wherein said contacting said tagged molecule with said universal detector is carried out in the presence of a development reagent binding moiety to generate a functional development reagent, further wherein said measuring hybridization of said identifier tag to said complementary detection probe includes measuring the contribution of said functional development reagent to the signal being measured.

21. (Original) The method of Claim 20, wherein said development reagent binding moiety comprises an oligonucleotide complementary to said development reagent.

22. (Original) The method of Claim 21, wherein said development reagent binding moiety is attached to at least one said detection probe.

23. (Original) The method of Claim 12, wherein said utilizing said second 5' flap as a reagent in at least one subsequent reaction to generate at least one tagged molecule comprises using said second 5' flap is used as a polymerization primer for rolling circle (RC) amplification of at least one circular oligonucleotide, said circular oligonucleotide comprising sequence complementary to said second 5' flap and sequence complementary to an identifier tag chosen to serve as an identifier for said target nucleotide sequence, such that said RC amplification generates at least one tagged molecule comprising multiple copies of said identifier tag.

24. (Original) The method of Claim 23, wherein said method is used to determine one or more polymorphic nucleotides in a single nucleotide polymorphism (SNP).

25. (Original) The method of Claim 23, wherein said method is used to detect a plurality of target nucleotide sequences in said sample.

26. (Original) The method of Claim 23, wherein said tagged molecule is trimmed to generate shorter tagged molecules comprising one copy of said identifier tag.

27. (Original) The method of Claim 12, further comprising transcribing said second 5' flap to generate RNA tagged molecules, wherein said method comprises:

a) providing at least one distinct second 5' flap comprising sequence complementary to the identifier tag chosen to serve as the identifier for each said target nucleotide sequence in said sample;

b) contacting each said distinct second 5' flap with a template comprising a double stranded RNA polymerase promoter and a single stranded portion of sequence complementary to said second 5' flap;

c) allowing each said second 5' flap to anneal to said sequence complementary to said second 5' flap;

d) ligating each said annealed second 5' flap to contiguous sequence;

e) transcribing each said annealed second 5' flap in the presence of RNA polymerase, generating at least one RNA tagged molecule comprising the identifier tag for each said target nucleotide sequence in said sample; and

f) contacting said at least one tagged RNA molecule with a universal detector comprising at least one complementary detection probe coupled to a detection means, wherein each said complementary detection probe comprises sequence complementary to each said identifier tag, and measuring hybridization of each said identifier tag to each said complementary detection probe, wherein said hybridization indicates the presence of the corresponding target nucleotide sequence in the sample.

28. (Original) The method of Claim 27, wherein said template comprising a double stranded RNA polymerase promoter and a single stranded portion of sequence complementary to said second 5' flap is a hairpin cassette.

29. (Original) The method of Claim 28, wherein said hairpin cassette is addressably labelled, such that said labelled hairpin cassette can be manipulated through a label-binding moiety.

30. (Original) The method of Claim 29, wherein said hairpin cassette is biotinylated.

31. (Original) The method of Claim 30, wherein said biotinylated hairpin cassette is contacted with streptavidin coupled to a solid support, such that said biotinylated hairpin cassette can be removed from the reaction mixture.

32. (Original) The method of Claim 27, wherein said method is used to determine one or more polymorphic nucleotides in a single nucleotide polymorphism (SNP).

33. (Original) The method of Claim 27, wherein said method is used to detect a plurality of target nucleotide sequences in said sample.

34. (Original) The method of Claim 12, further comprising transcribing said second 5' flap to generate RNA tagged molecules, wherein said method comprises:

a) providing at least one distinct second 5' flap corresponding to each said target nucleotide sequence in said sample;

b) contacting each said distinct second 5' flap with at least one single stranded template, wherein each template comprises a portion of sequence complementary to one said distinct second 5' flap, a portion of sequence encoding one strand of RNA polymerase promoter, and at least one copy of the identifier tag chosen to

serve as the identifier for said target nucleotide sequence corresponding to said distinct second 5' flap;

c) allowing each said second 5' flap to anneal to said template sequence complementary to said second 5' flap, forming a double-stranded DNA polymerase binding site;

d) generating a double stranded copy of said template by DNA polymerase binding to said double stranded site and extension of said annealed second 5' flap;

e) contacting said double stranded copy of said template with RNA polymerase, allowing RNA polymerase to transcribe sequence downstream of said RNA polymerase promoter, thereby generating RNA tagged molecules comprising the identifier tag for each said target nucleotide sequence in said sample; and

f) contacting said at least one tagged RNA molecule with a universal detector comprising at least one complementary detection probe coupled to a detection means, wherein each said complementary detection probe comprises sequence complementary to each said identifier tag, and measuring hybridization of each said identifier tag to each said complementary detection probe, wherein said hybridization indicates the presence of the corresponding target nucleotide sequence in the sample.

35. (Original) The method of Claim 34, wherein said method is used to determine one or more polymorphic nucleotides in a single nucleotide polymorphism (SNP).

36. (Original) The method of Claim 34, wherein said method is used to detect a plurality of target nucleotide sequences in said sample.

37-91 (Canceled)

92. (New) A method of detecting a target nucleotide sequence in a sample comprising:

obtaining a sample comprising template, said template comprising at least one target nucleotide sequence;

obtaining an oligonucleotide probe comprising a 3' portion complementary to a portion of said template comprising target nucleotide sequence and a 5' portion not substantially complementary to a portion of said template comprising target nucleotide sequence;

obtaining an upstream oligonucleotide complementary to a portion of said template comprising target nucleotide sequence, wherein said portion is 3' to and partially overlapping the 3' portion of said template comprising target nucleotide sequence complementary to said probe nucleic acid;

contacting said sample with said oligonucleotide probe and said upstream oligonucleotide under conditions which permit hybridization of said oligonucleotide probe and said upstream oligonucleotide to said template comprising target nucleotide sequence, whereby the 5' portion of said oligonucleotide probe forms a 5' flap that is not substantially complementary to a portion of said template comprising target nucleotide sequence;

cleaving said 5' flap from said oligonucleotide probe; and
detecting said cleaved 5' flap.

93. (New) The method of Claim 92, wherein multiple probe oligonucleotides are cleaved and multiple 5' flaps are released.

94. (New) The method of Claim 92, wherein an endonuclease mediates said cleaving step.

95. (New) The method of Claim 94, wherein said endonuclease comprises a 5' endonuclease.

96. (New) The method of Claim 92, wherein the step of detecting said cleaved 5' flap comprises using said cleaved 5' flap as a primer in a rolling circle amplification procedure.

97. (New) The method of Claim 92, wherein said cleaved 5' flap comprises a promoter or a sequence complementary to a promoter and the step of detecting said cleaved 5' flap comprises initiating transcription from said promoter.